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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/092,598	03/08/2002	Jose Cibelli	60141.0068USU1	1067

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EXAMINER	
BERTOGLIO, VALARIE E	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 04/12/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/092,598	CIBELLI, JOSE	
	Examiner	Art Unit	
	Valarie Bertoglio	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-72 is/are pending in the application.

4a) Of the above claim(s) 8-20,28-50,52-68 and 70-72 is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-7,21-25,51 and 69 is/are rejected.

7)☒ Claim(s) 24 is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 08 March 2002 is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of Group I in the reply filed on 01/13/2005 is acknowledged. The traversal is on the ground(s) that it would not require undue burden to search Groups I-III together as a search of genetic engineering is likely to uncover art including knockout, gene insertion and RNA interference. This is not found persuasive because while the search of the broad class of genetic engineering may reveal several different methods of genetic engineering and may present useful information, a separate search would be required to perform a thorough search for cloning using antisense technology. Furthermore, the methods of each of the inventions are materially differently and independent and require different technical considerations. The embryos made by the different methods are structurally distinct.

The requirement is still deemed proper and is therefore made FINAL.

Claims 8-20,28-50,52-68 and 7-72 are withdrawn as being drawn to a non-elected invention. Claims 1-72 are pending and claims 1-7,21-27,51 and 69 are under consideration in the instant office action.

Claim Objections

Claim 24 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 24 is directed to the cell lineage deficient embryonic stem cells derived from the inner cell mass of claims 22. Claim 24 fails to further limit the subject

matter of the base claim. Claim 22, which depends from base claim 1, is a method of making nuclear transfer embryos and is not directed to inner cell mass cells.

Claim Rejections - 35 USC § 112-1st paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-7, 21-27,51 and 69 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Enablement is considered in view of the Wands factors (MPEP 2164.01(a)). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (*Wands*, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (*Wands*, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the

breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

The claims are drawn to a method of making a nuclear transfer embryo that is incapable of differentiating into a particular lineage by isolating a mammalian differentiated cell, genetically engineering said cell, and transferring the nucleus from the genetically engineered cell into a recipient cell to form a nuclear transfer embryo. The claims recite methods of modifying the nuclear genome of a somatic cell at an endogenous locus by a gene targeting event mediated by homologous recombination. The claims broadly encompass use of any type of somatic donor cell as well as any type of recipient cell. The elected invention is specifically drawn to genetically modifying the donor cell by disrupting the function of a gene by gene targeting and the claims encompass knocking out any gene in a somatic cell such that the cell is incapable of differentiating into a particular cell lineage. Some dependent claims limit aspects of the claimed method. Claim 21 limits the recipient cell to mammalian oocytes or ES cells. Claims 25-27 limit the gene being knocked out to specific genes or sets of genes. The claims broadly encompass knocking out a gene in a neural cell and transplanting the nucleus into a hepatocyte.

The specification provides only prophetic teachings with respect to the claimed methods. The specification prophetically discusses disruption of a gene in differentiated somatic cells wherein the gene is necessary for development of a particular lineage followed by transfer of the nucleus into donor cells. The specification does not provide any working examples or any guidance with respect to what cell types to use as donors, to what cell types to use as recipients or what genes, when knocked out, would be effective in accomplishing the claimed invention. Paragraph 0043 of the specification purports that any gene expressed specifically in a specific

lineage lineage can be knocked out to cause "de-differentiation" of the respective lineage. The specification lists a number of examples of such genes but fails to provide any guidance as to what the genes do or what effect the gene knockout, if any, will have. The specification fails to provide any guidance, whatsoever, as to how to genetically engineer any gene at all so as to prevent formation of a particular lineage.

The claims are not enabled by the instant specification because they require that a gene be knocked out to inhibit the development of a particular lineage. The specification fails to provide any guidance as to what genes one might target in inhibiting a particular lineage. There are a number of parameters to be considered in selecting a gene for targeting in the instant invention. First, it must be known what genes will inhibit differentiation of a particular desired lineage. Second, the genes should not directly affect other lineages, particularly the differentiated tissue type desired as a final product. Third, indirect effects of lineage loss on the desired differentiated lineage must be considered. For example, the development of neural tissue from ectoderm requires signaling from underlying mesoderm [see **Developmental Biology,** 5th edition, Sinauer Associates, Inc. Publishers, ed. Scott Gilbert, pages 619-624]. Therefore, inhibition of the mesodermal lineage would affect development of neural tissue from ectoderm. The specification does not suggest a single gene to be knocked out to inhibit any particular lineage and allowing appropriate differentiate of other cell types that would be useful. It would be highly unpredictable what effects any particular gene knockout would have on lineage development in a resultant NT embryo.

The unpredictability of the effects of gene knockout is not only dependent upon the characteristics of the gene but also upon other yet uncharacterized effects of the nuclear transfer

process. In experiments for the production sheep comprising a disruption of the α1,3-galactosyltransferase gene, live births were achieved but the animals died within two weeks of birth [Denning, **Cloning and Stem Cells**, 3:221-231, (2001), specifically see page 230, col. 1, parag. 2, lines 1-8]. This was unexpected. However, Denning reports that McCreath achieved live birth and survival of two gene targeted sheep with disruptions in different genes (page 230, col. 1, parag. 2, lines 9-12). Denning analyzed the results of both sheep experiments and arrived at the conclusion that it is possible that for gene targeted sheep, the success depends on unknown factors. However, Denning notes, the use of fibroblasts to produce gene-targeted pigs is not possible (page 230, col.1, parag. 1, lines 7-13). Denning continues by stating that for sheep the parameters of cell growth and targeting efficiency reported therein just about make feasible the production of gene targeted sheep. In other words, at the time of filing, the skilled artisan would have regarded the production of gene targeted sheep and pigs as being unpredictable requiring an undue amount of experimentation without a predictable degree of success. Since the art at the time of filing clearly indicated that method for producing two gene targeted livestock animals were unpredictable, it would be reasonable to extend the unpredictability to the genus "mammal" absent evidence to the contrary.

The instant specification suggests a number of cell types as nuclear donors [see p. 10-11, paragraph 0023], including neural, fibroblast, endothelial, cardiac, esophageal, stomach, lymphocytes and red blood cells, to name a few. The art has taught that somatic cells that have been shown to support nuclear transfer are usually of fibroblast origin, although nuclear transfer has been shown to be successful in some species of mammals using cumulus, oviduct and mammary oviduct and granulosa cells, cell types that cannot be maintained long enough in

culture to establish a cell line through gene-targeting via homologous recombination. The unpredictability of homologous recombination in somatic cells is supported by the art. Thomson *et al.* [**Reprod. Supp.**, 61:495-508 (2003)] review the state of the art of gene targeting in somatic cells for use in nuclear transfer methodologies and state that procedures to enhance the lifespan of targeted somatic cells *in vitro* are needed. In particular, Thomson states that premature senescence often occurs, which makes it difficult to confirm a targeting event in somatic cells and that cloning efficiency has been negatively correlated with passage number. See pp. 501. The inefficiency and unpredictability of homologous recombination in somatic cells is supported by Polejaeva and Campbell [**Theriogenology**, 53:117-126 (2000)] who teach that gene targeting in somatic cells is unpredictable because of the lower frequency of homologous recombination than ES cells and the finite number of cell divisions. Polejaeva and Campbell further discuss specific criteria for more efficient somatic cell gene targeting, such as the ability of the cells to have a high single cell-cell cloning efficiency because during drug selection, the cells must be able to expand into clonal cultures. However, they note that human dermal fibroblasts are not able to proliferate under regular culture conditions, and thus, optimization of culture conditions must be attained for success in somatic cell gene targeting. See p. 120-121. Clark [**Transgenic Research,** 9:263-275 (2000)] teaches that only primary somatic cells have been used successfully in gene targeting experiments to produce livestock having a disrupted gene of choice (page 265, col.2, parag. 1, lines 12-15). In addition, Clark teaches that about 45-population doublings are required to generate targeted cells (page 268, col. 2, parag. 1, lines 1-5). Denning teaches that primary cells have limited proliferation capacity and any genetic modifications and nuclear transfer must be accomplished prior to senescence, specifically refer

to page 222, col. 1, lines 5-8]. In a study of sheep and goat primary somatic cells, Denning found that of primary somatic cells, fibroblasts were the only cells that either grew at all from the primary cell source or has sufficient population doublings for the selection required in targeted gene transfer. Sheep primary cell cultures primarily were composed of fibroblasts after the third passage or about 12 doublings (Denning, page 224, col. 2, lines 11-13). Further, a comparison of separate Black Welsh sheep primary cell fibroblast cultures showed vast differences in the number of doublings prior to senescence; 110 doublings versus 40 doublings (Denning, page 224, col. 2, lines 16-19). In a similar analysis of pig primary cultures, fibroblasts, as in the sheep study, became the predominant cell-type after three passages, but, unlike sheep, pig fibroblasts underwent a crisis after 40 population doublings and had an unstable karyotype (Denning, page 224, col. 2, parag. 4 line 4 to page 225, col. 1, line 8). Additional studies of cell cultures prepared from fetal pig organs (gut, kidney, lung and mesonephros) showed that these cells senesced or entered crisis after even fewer doublings than the fibroblast cultures (page 225, col. 1-2, bridg. sent.). The art further taught at the time of filing, that the even if sufficient population doublings could be achieved for selection, many of the pure sheep targeted clones senesced before they could be expanded for nuclear transfer, meaning that targeting frequency was lower than expected (page 228, col. 1-2, bridg. sent.). Similar experiments in pigs demonstrated that all the clones senesced, and no targeted cells for nuclear transfer were obtained. Clearly, the art supports the unpredictability in the gene targeting using any somatic cell type for use in nuclear transfer methodologies, and more specifically, that candidate somatic cells that would be used for gene targeting must be able to survive multiple rounds of cell division, selection and overcome senescence. The specification fails to provide teachings or guidance for utilizing any

somatic cell for gene targeting which would be further used in nuclear transfer methods. While the state of the art supports that particular cell types, such as fetal fibroblasts, can be used in the claimed methods, specific guidance must be provided to enable the breadth of the claims.

The claims encompass methods of nuclear transfer utilizing any recipient cell type with any nuclear donor; however, it is noted that successful nuclear transfer requires both ideal recipient oocytes and donor cells. A nuclear transfer embryo does not result from use of any cell type other than an oocyte as the recipient cell (specifically refer to claim 21). The specification teaches that stem cells may not make all the factors necessary for reprogramming that oocytes are known to have (page 4, paragraph 0008). Thus, the only cell type that would be a "suitable recipient", as required by claim 1, is an oocyte. Campbell [**Cloning & Stem Cells**, 3(4):201-208 (2001)] has taught, "Successful development [of the nuclear transfer unit] is dependent upon numerous factors, including type of recipient cell, source of recipient cell, method of reconstruction, activation, embryo culture, donor cell type, and donor and recipient cell cycle stages." See *Abstract*. Campbell teaches that metaphase II [MII] oocytes are considered the cytoplast of choice because the genetic material is arranged upon the meiotic spindle and easily removed [see p. 202, 2nd column, 1st ¶], further, following introduction of the donor somatic cell into an enucleated oocyte, activation must occur to induce further development and the timing of this activation in relation to nuclear transfer has been implicated in the ability of the nuclear transfer unit to develop further [see p. 203, 2nd col.]. Fulka *et al.* [**Theriogenology**, 55(6):1373-1380 (2001)] state that the three basic types of cytoplasts 1) enucleated metaphase II oocytes that are used immediately for nuclear transfer, 2) MII oocytes that are enucleated and aged in culture to be used in nuclear transfer and 3) oocytes that are first activated and then enucleated in

telophase II before use in nuclear transfer. See p. 1374. Accordingly, the state of the art supports that only oocytes in MII or oocytes in telophase II be used for successful nuclear transfer. While prophetically stating that any stage oocyte can be used as a donor, the specification fails to teach or provide guidance for any donor cell other than MII or telophase II oocytes.

The claims also encompass cross-species nuclear transfer (specifically claim 21), which is contemplated by the specification [specifically see page 20, paragraph 0041]. Cross-specie nuclear transfer, at the time of filing, was unsuccessful. Meirelles demonstrates that methods of nuclear transfer where the nuclear material of *Bos indicus* is inserted into the oocyte of *Bos taurus* produces calves comprising the nuclear material of *Bos indicus* and the mitochondria of *Bos Taurus*[**Genetics,** 158:351-356 (2001)]. Meirelles *et al.* teach that previous attempts to use the *Bos* oocyte as hosts for nuclear transfer from unrelated species allowed development to the blastocyst stage, and conclude that incompatibility among the nuclear and mitochondrial genetic systems is responsible for the early arrest. Meirelles *et al.* also point to similar failures using *Mus caroli* and *Mus musculus* citing Dominko *et al.* Meirelles *et al.* conclude that in light of their results and the failures of the prior art, that nuclear transfer across subspecies barriers is possible (pp. 351-355). Applicant's claims encompass nuclear transfer (cloning) when the nucleus is of one species and the oocyte is of another species. This clearly lacks predictability given the teachings of Meirelles.

Therefore, in light of the lack of guidance in the specification with respect to what genes to genetically alter, with respect to what cell types to use as a nuclear donor, and with respect to what a suitable recipient cell would be and in light of the state of the art with respect to the

unpredictability of phenotypes in gene KO animals and nuclear transfer animals, the underdeveloped art of gene targeting in somatic cells other than fibroblasts, and the limited number of suitable recipient cells for nuclear transfer, it would require undue experimentation for one of ordinary skill in the art to carry out the invention as claimed.

Claim Rejections - 35 USC § 112-2nd paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3,22,24 and 51 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 3 recites the limitation "said...cells derived therefrom" in lines 1-2. There is insufficient antecedent basis for this limitation in the claim. The phrase "said blastula or morula, or cells derived from said blastula or morula" is suggested. Claim 51 depends from claim 3.

Claim 22 recites the limitation "said blastocyst or morula" in line 1. There is insufficient antecedent basis for this limitation in the parent claim. Claim 24 depends from claim 22.

Claim 51 recites the limitation "Differentiated cells" in line 1. There is insufficient antecedent basis for this limitation in the claim.Claim 51 depends from claim 3, which depends from claim1. Claims 1 and 3 refer to 3 different differentiated cells. It is unclear to which differentiated cells claim 51 is referring.

Claim Rejections - 35 USC § 102

> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

1) Claim 51 is rejected under 35 U.S.C. 102(b) as being anticipated by Braun, [**EMBO J.**, 15: 310-318 (1996)].

Claim 51 is a product by process claim in which the process of creating the animal carries little patentable weight. It is only the product, which is anticipated by the prior art and not the process by which the product was made. This is because the final product (differentiated cells) is not distinguished by any particular features or characteristics resulting from the process by which it is made. As such, the limitations of the claimed differentiated cells are met by any differentiated cells in the prior art. Patentability of a product-by-process claim is determined by the novelty and nonobviousness of the claimed product itself without consideration of the process for making it, which is recited in the claims. *In re* Thorpe, 227 USPQ 964 (Fed. Cir. 1985).

Braun taught the differentiation ES cells comprising gene-targeted modifications of the myoD and myf-5 genes (see abstract).

Thus, the teachings of Braun anticipate the limitations of claim 51.

2) Claim 24 is rejected under 35 U.S.C. 102(b) as being anticipated by Mishina [**Genes and Development,** 9:3027-3037 (1995)].

Claim 24 is drawn to a cell lineage deficient embryonic stem cell derived from the inner cell mass cells of claim 22. The claim is unclear because parent claim 22 is a unclear method claim rejected above under 35 USC 112, 2nd paragraph. As a result, claim 24 is also unclear. It appears that the embryonic stem cells of claim 24 are not distinguished by any particular features or characteristics and would not differ from any other cell lineage deficient embryonic stem cells known in the art.

Mishina taught making BMPR knockout mice by targeted disruption of the BMPR gene in mouse ES cells and using the ES cells to generate mice. The homozygous mutant embryos died in utero and were found to lack mesoderm. Therefore, the ES cells used to make the mice are lineage deficient in that they are not capable of directing the formation of mesoderm.

Thus, the teachings Mishina anticipate the limitations of claim 51.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Valarie Bertoglio whose telephone number is (571) 272-0725. The examiner can normally be reached on Mon-Thurs 5:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Valarie Bertoglio
Examiner
Art Unit 1632

Scott D. Priebe

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